

Amendments to the Specification

Please amend the paragraph beginning at page 8, line 37, as follows:

In an aspect, the invention also provides attenuated live virus CMV vaccines wherein at least one open reading frame of a Toledo genomic region is replaced by a segment of Towne genome which is not present in AS169. The Towne genome comprises a region not present in AD169; the region contains open reading frame designated UL147, UL152, UL153, and UL154 and generally is spanned by nucleotides 178221 to 180029 of the Towne genome according to the AD169 (EMBL accession number X17403) numbering convention. An attenuated virus of the invention can, in one embodiment, comprise a Toledo genome wherein the Toledo genome region spanning open reading frames UL133 to UL151 are replaced with a Towne genome region spanning UL147, UL152, UL153, and UL154; this engineered CMV virus variant is an attenuated Toledo virus which comprises desirable features of Towne while reducing undesirable virulence of the Toledo genome region. The invention provides other variations of this basic method, whereby a segment of the Toledo genome region comprising at least one open reading frame is deleted or otherwise structurally disrupted in a CMV variant having a Toledo genome region or its homolog, and a segment of a Towne genome region comprising at least one open reading frame is inserted in the CMV variant. In an embodiment, the engineered CMV variant comprises: (1) Toledo DNA (DNA substantially identical to a Toledo strain, preferably identical to it) from about nucleotides 1 to about 168,000 corresponding to (i.e., according to) the AD169 nucleotide (EMBL accession number X17403) numbering convention, operably linked to (2) Towne DNA (DNA substantially identical to a Towne strain, preferably identical to it) from about nucleotides 143,824 to 189,466 according to the AD169 nucleotide (EMBL accession number X17403) numbering convention, operably linked to (3) Toledo DNA (DNA substantially identical to a Toledo strain, preferably identical to it) from about nucleotides 189,466 to about 209,514 corresponding to (i.e., according to) the AD169 nucleotide (EMBL accession number X17403) numbering convention, operably linked to (4) Towne DNA (DNA substantially identical to a Towne strain, preferably identical to it) from about nucleotides 200,080 to 229,354 according to the AD169 nucleotide (EMBL accession number X17403) numbering convention. The invention also provides vaccine compositions and formulations of such attenuated CMV viruses, which can include adjuvants, delivery vehicles, liposomal formulations, and the like. The invention also provides the use of such attenuated CMV

variants for prevention of CMV disease and infection; in one aspect this use includes administration of such vaccine to human subjects.

Please amend the paragraph beginning at page 14, line 15, as follows:

Figure 5. CMV Towne and Toledo cosmids used to regenerate specific chimeric CMV viruses. The location of the cosmid insert are indicated beneath the appropriate viral genome. The numbers at the end of the insert denote the endpoints determined by DNA sequence analysis; the numbers correspond to AD169 genomic sequence in GenBank (EMBL accession number X17403). "XXX" " denotes an end which was refractory to DNA sequence analysis. These ends were mapped by restriction enzyme and Southern blot analyses. The vertical dashed line represents the location of the internal "a" sequence of the virus. The lower line depicts the structure of the Tol/Twn 39/50 genome. The thick gray line denotes sequences derived from Toledo and the thin black line depicts sequences contributed from highly-passaged Towne strain. Regions of overlap could be derived from either virus and are represented by a region of a thick gray and a thin black line together. The Tol/Twn 39/50 genome does not contain the Toledo genomic region.

Please amend the paragraph beginning at page 32, line 18, as follows:

In an aspect, the invention also provides attenuated live virus CMV vaccines wherein at least one open reading frame of a Toledo genomic region is replaced by a segment of Towne genome which is not present in AS169. The highly-passaged Towne genome comprises a region not present in AD169; the region contains open reading frame designated UL147, UL152, UL153, and UL154 and generally is spanned by nucleotides 178221 to 180029 of the Towne genome according to the AD169 (EMBL accession number X17403) numbering convention. An attenuated virus of the invention can, in one embodiment, comprise a Toledo genome wherein the Toledo genome region spanning open reading frames UL133 to UL151 are replaced with a Towne genome region spanning UL147, UL152, UL153, and UL154; this engineered CMV virus variant is an attenuated Toledo virus which comprises desirable features of Towne while reducing undesirable virulence of the Toledo genome region. The invention provides other variations of this basic method, whereby a segment of the Toledo genome region comprising at least one open reading frame is deleted or otherwise structurally disrupted in a CMV variant having a Toledo genome region or its homolog, and a segment of a Towne genome region comprising at least one open reading

frame is inserted in the CMV variant. In an embodiment, the engineered CMV variant comprises: (1) Toledo DNA (DNA substantially identical to a Toledo strain, preferably identical to it) from about nucleotides 1 to about 168,000 corresponding to (i.e., according to) the AD169 nucleotide (EMBL accession number X17403) numbering convention, operably linked to (2) Towne DNA (DNA substantially identical to a Towne strain, preferably identical to it) from about nucleotides 143,824 to 189,466 according to the AD169 nucleotide (EMBL accession number X17403) numbering convention, operably linked to (3) Toledo DNA (DNA substantially identical to a Toledo strain, preferably identical to it) from about nucleotides 189,466 to about 209,514 corresponding to (i.e., according to) the AD169 nucleotide (EMBL accession number X17403) numbering convention, operably linked to (4) Towne DNA (DNA substantially identical to a Towne strain, preferably identical to it) from about nucleotides 200,080 to 229,354 according to the AD169 nucleotide (EMBL accession number X17403) numbering convention. The invention also provides vaccine compositions and formulations of such attenuated CMV viruses, which can include adjuvants, delivery vehicles, liposomal formulations, and the like. The invention also provides the use of such attenuated CMV variants for prevention of CMV disease and infection; in one aspect this use includes administration of such vaccine to human subjects.

Please amend the paragraph beginning at page 41, line 15, as follows:

Plaques derived from the chimeras were allowed to grow until 100% of the monolayer exhibited CPE. At this point, DNA was extracted from the supernatant and cellular fractions and analyzed by restriction enzyme digestion. The structures of the viruses can be deduced by the cosmids used for construction of the chimera and confirmed by comparing the EcoRI digestion pattern to the maps derived for Towne and Toledo (see Fig. 10). Table 2 describes the composition of each of the chimeras, the nucleotide limits are derived from sequence analysis of the end of each cosmid insert and its homology to the AD169 strain of HCMV, which has been sequenced in its entirety (EMBL accession number X17403). All of the chimeras had restriction enzyme patterns consistent with the proposed structures.

Amendments to the Sequence Listing

Please delete the previously submitted Substitute Sequence Listing in its entirety and enter the attached Second Substitute Sequence Listing.